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Inhibition of Hypothalamic LHRH Depletion after Ovariectomy by Transplantable Prolactin and Growth-Hormone-Secreting Tumors (41181)

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Abstract. Anestrous Wistar–Furth rats bearing implants of the Furth MtTW15 tumor and diestrous tumor-free rats were ovariectomized and serum luteinizing hormone (LH), follicle-stimulating hormone (FSH) concentrations, and hypothalamic luteinizing hormone-releasing hormone (LHRH) content, were studied. The increase of LH and FSH, after ovariectomy, was significantly greater in tumor-free rats than in rats bearing prolactin secreting tumor implants. The hypothalamic LHRH content of the intact tumor-bearing rats was greater ($P < 0.01$) than that of tumor-free rats. Furthermore, the depletion of hypothalamic LHRH content was greater in tumor-free rats than in tumor-bearing rats. These results suggest that this prolactin and growth-hormone-secreting tumor prevents gonadotropin release by reducing LHRH release from the hypothalamus.

Hyperprolactinemia is associated with reduced gonadotropin secretion in a variety of species including the rat. The Furth MtTW15 tumor which secretes both prolactin and growth hormone makes rats hyperprolactinemic and its presence can largely prevent secretion of luteinizing hormone (LH) in response to gonadectomy (1, 2). Because we found the luteinizing hormone-releasing hormone (LHRH) content of the hypothalamus was elevated in male rats bearing this tumor, we suggested that LHRH release was reduced (2). In the present study, this hypothesis was tested when negative feedback was reduced following ovariectomy. Release of LHRH from the hypothalamus was judged by measuring hypothalamic LHRH content and serum gonadotropins at 7 and 14 days after surgery.

Materials and Methods. Adult female Wistar–Furth rats (200–225 g, Harlan Sprague–Dawley Co.) were used for this study. Rats were housed in a 14- to 10-hr light–dark cycle in a temperature-controlled room (25 ± 1°). Wayne Lab Blox and water were provided *ad libitum*. Vaginal smears were taken daily, and at weekly intervals the rats were weighed. All blood sample collection, animal surgery, and tissue collection was performed between 1500 and 1800 hr. The Furth MtTW15 tumor was transplanted subcutaneously over the backs of the rats (3).

Four weeks later, a 2.0-ml blood sample was taken by orbital sinus puncture under ether anesthesia from both the tumor-bearing rats and diestrous tumor-free rats which had exhibited regular 4-day estrous cycles (PEMD). Serum LH, FSH, prolactin, and progesterone concentrations were determined later by RIA and were used to confirm the endocrine status of tumor bearing rats.

Groups of anestrous tumor-bearing rats (rats which had exhibited constant diestrous smears for 2 weeks which had gonadotropin and progesterone concentrations below those of diestrous tumor-free rats) [see Table 1] and diestrous tumor-free rats were ovariectomized. Seven or fourteen days after ovariectomy, the ovariectomized rats and control groups of intact anestrous tumor-bearing and diestrous tumor-free rats were killed by decapitation. The trunk blood was collected for LH and FSH assay. The brains were collected and quick frozen on dry ice. Brains were stored at −60° until they were homogenized for LHRH assay. The uterus and ovaries of the intact rats

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were weighed and examined at the time of decapitation. Trunk blood was allowed to clot for 24 hr at 4°, the clots were removed, and the serum was centrifuged at 2000*g* to remove the remaining cells. The serum was then snap frozen and stored at −20° until RIAs were performed.

Serum progesterone was measured by the methods of Resko *et al.* (4) and Louis *et al.* (5) using antiserum No. 337-anti-progesterone-11-BSA provided by Dr. Gordon Niswender (Department of Physiology and Biophysics, Colorado State University, Ft. Collins, Co.). Progesterone (Sigma Chemical Co.) was used as the reference preparation, and progesterone (1,2,6,7-3H progesterone, New England Nuclear) was used as the labeled antigen and for determination of extraction efficiency. Free and bound steroids were separated with dextran charcoal (6).

Serum LH, FSH, and prolactin were measured by radioimmunoassay (RIA) using NIAMDD kits. Assays were performed by the double-antibody method using the instructions enclosed with the kits.

The hypothalamus was dissected from the frozen brain as follows: cuts were made anterior to the optic chiasma, at the lateral hypothalamic sulci, and just anterior to the mamillary body. This tissue block was then trimmed at a depth of 5 mm. The hypothalamus was placed in 2 ml of cold 0.1 N HCL and homogenized for 60 sec by sonification. The homogenate was removed and neutralized with 2 ml of 0.1 N NaOH (7). LHRH was measured by the method of Nett *et al.* (8, 9) using antiserum R-42 (provided by Dr. Terry Nett, Department of Physiology and Biophysics, Colorado State University). Synthetic LHRH (Calbiochem) was used as the reference preparation and hormone for iodination. Iodination was done with chloramine-T as outlined by Nett *et al.* (8, 9).

Samples for RIAs were run in duplicate in individual progesterone, prolactin, LHRH, LH, and FSH assays. The coefficients of intraassay variation for the various assays were: nine percent for progesterone, 9% for prolactin, 6% for LHRH, 11% for LH, and 7% for FSH as calculated from duplicate samples (10). Factorial analysis of variance was used to analyze effects of ovariectomy. Multiple comparisons of means were analyzed by the Student–Newman–Keuls test or, if indicated, individual comparisons by Student's *t* test (11).

Results. One month after tumor implantation, significant reductions in serum concentrations of LH, FSH, and progesterone were evident in the tumor-bearing rats as compared to the diestrous tumor-free rats (Table 1). These tumor-bearing rats were either left intact for an additional 7 days or were ovariectomized and killed 7 or 14 days later.

At the time of ovariectomy, there were no signs of uterine enlargement or ballooning in the tumor-bearing rats. Furthermore, none of the rats had exhibited an estrous vaginal smear for at least 2 weeks. The uterine and ovarian weights of the intact tumor-bearing rats were significantly lower than those in diestrous tumor-free rats (Table 1).

Hypothalamic LHRH content (Fig. 1) of intact tumor-bearing rats was significantly greater than that of tumor-free rats, similarly at 7 and 14 days after ovariectomy, hypothalamic LHRH content was greater in tumor-bearing rats ($P < 0.01$). In tumor-bearing rats the decrease in hypothalamic LHRH content was delayed for 7 days after ovariectomy.

Serum LH and FSH release after ovariectomy (Figs. 2 and 3) was significantly reduced in the tumor-bearing animals as compared to the tumor-free animals at both 7 and 14 days after ovariectomy ($P < 0.01$).

Discussion. The present study suggests that the Furth MtTW15 tumor inhibits the release of LHRH from the hypothalamus. This tumor resulted in increased hypothalamic LHRH content in intact anestrous rats. After ovariectomy, the tumor delayed the decrease in hypothalamic LHRH content and largely prevented concomitant increases in serum LH and FSH concentrations.

We believe that reduction of hypothalamic LHRH content after ovariectomy reflects increased release of LHRH from the hypothalamus and that experimental manipulations which elevate hypothalamic LHRH

TABLE I. SERUM HORMONE LEVELS OF INTACT DIESTROUS TUMOR-FREE RATS AND TUMOR-BEARING RATS 1 MONTH AFTER TUMOR IMPLANTATION, AND UTERINE, OVARIAN, AND BODY WEIGHTS OF RATS WHEN KILLED

Group	LH (ng/ml)	FSH (ng/ml)	Prolactin (ng/ml)	Progesterone (ng/ml)	Uterus (mg)	Paired ovaries (mg)	Body weight (g)
Diestrous tumor-free rats	36.2 ± 5.1[a] (20)[b]	522 ± 23 (20)	19.6 ± 3.2 (20)	8.8 ± 1.3 (20)	337 ± 12.6 (10)[c]	74 ± 3.0 (10)[c]	292 ± 5 (30)
Tumor-bearing rats	11.9 ± 1.1** (36)	463 ± 27 (36)	4931 ± 297** (36)	6.1 ± 2.1 (36)	262 ± 11.4 (10)	56 ± 2.3 (10)	206 ± 2 (30)

Note. Serum and tissue samples were taken between 1500 and 1800 hr.
[a] Mean ± SEM. Significant difference from diestrous tumor-free rats by Student's *t* test, * $P < 0.05$ or ** $P < 0.01$.
[b] Number of rats per group.
[c] Intact rats only.

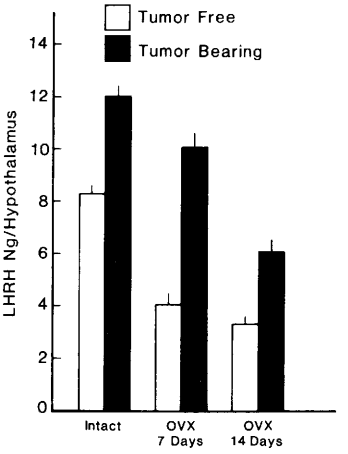

FIG. 1. Hypothalamic LHRH content (mean ± SEM) of tumor-free and tumor-bearing rats either intact or ovariectomized (OVX) for 7 or 14 days ($N = 10$, mean ± SEM).

content in ovariectomized rats, do so by suppressing LHRH release. This interpretation is supported by several reports in the literature; for example, there is a decrease in hypothalamic LHRH content preceding the proestrus gonadotropin surge (12, 13). Furthermore, LHRH is increased in the hypophyseal portal veins (14, 15) and in the general circulation on the afternoon of proestrus. Several studies have shown that hypothalamic LHRH content is decreased as gonadotropins rise (16–19) after gonadectomy. Accompanying this decrease in hypothalamic LHRH stores, there

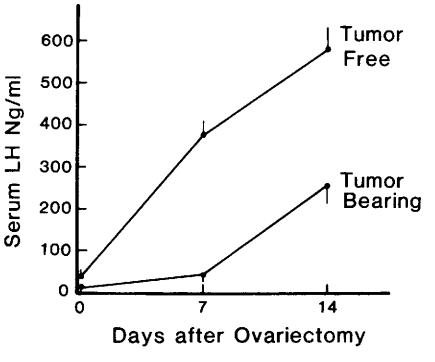

FIG. 2. Serum LH concentrations (ng/ml) between 1300 and 1500 hr of tumor-free and tumor-bearing rats either intact or ovariectomized for 7 or 14 days ($N = 10$, mean ± SEM).

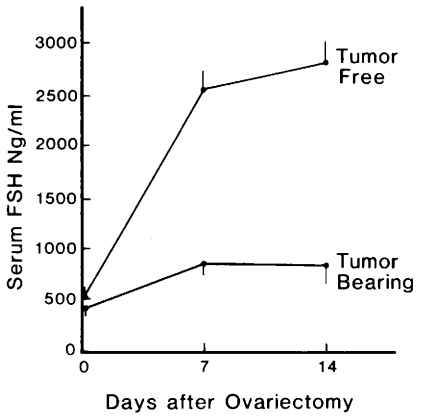

FIG. 3. Serum FSH concentrations (ng/ml) between 1300 and 1500 hr of tumor-free and tumor-bearing rats either intact or ovariectomized for 7 or 14 days. (N = 10, mean ± SEM)

is an increase in LHRH levels in the hypophyseal portal blood (20). An increase in LHRH concentrations in jugular vein blood has also been reported after the castration of sheep (21). The increase in serum LH and FSH after gonadectomy probably originates from increased LHRH secretion, because immunization against LHRH prevents the postcastration rises in serum and pituitary LH and FSH (22). In a similar manner, LHRH antiserum can prevent positive feedback release of LH in response to estradiol benzoate (19) or at proestrus (23).

The changes in hypothalamic LHRH content after gonadectomy probably originate from reduced negative feedback by the gonadal steroids (24), since it has been shown that estrogen or testosterone injections can prevent the increases in serum gonadotropins and reductions in hypothalamic LHRH content which take place after gonadectomy (16–19).

It is not likely that the Furth MtTW15 tumor inhibited gonadotropin release by stimulation of ovarian or adrenal steroid secretions. The tumor-bearing rats of this study had exhibited constant diestrous vaginal smears for at least 2 weeks before ovariectomy. Moreover, the uteri of these rats were not enlarged, and serum progesterone was below diestrous levels. Also, Seo *et al.* (25), have reported that serum estrogen and corticosterone levels were not increased in rats bearing implants of the growth-hormone-secreting (GH3) rat pituitary tumor. Bartke *et al.* (26), using pituitary gland grafts to make male rats hyperprolactinemic, found an increase in adrenal mass but no increase in serum corticosterone, estradiol, or progresterone concentrations. We have previously shown that there is no difference in LH and FSH release after castration and/or castration and adrenalectomy of male rats bearing implants of this tumor (2). These results suggest that the enlarged adrenals in these rats do not contribute to the inhibition of gonadotropin secretion.

Implants of the Furth MtTW15 tumor have been reported to cause hypothyroidism (27, 28). However, it has been reported that removal of the thyroid augments gonadotropin secretion in gonadectomized animals (29, 30). It is, therefore, unlikely that the observed changes in serum gonadotropins and hypothalamic LHRH were caused by altered thyroid activity.

It is likely that some neurotransmitter is involved in the suppression of LH secretion, because this tumor can also prevent growth hormone, TSH, and prolactin secretion by the *in situ* pituitary gland (27, 28, 31). Implants of the Furth MtTW15 tumor (32) or injections of prolactin (33) result in increased hypothalamic dopamine turnover, and increased dopamine turnover has been shown to reduce prolactin secretion by the *in situ* pituitary gland (31). Although it has been suggested that dopaminergic neurons might inhibit gonadotropin secretion (34), this point is not fully resolved. Alternatively, secretions of this tumor might act directly on the LHRH neurons of the hypothalamus to prevent LHRH release.

At present, we do not fully understand the mechanism by which the Furth MtTW15 tumor reduces gonadotropin secretion. This reduction apparently involves both reduced release of LHRH from the hypothalamus, as we have previously reported in male rats, and a reduction in pituitary responsiveness to LHRH (2). Chronic reduction of LHRH release from the hypothalamus and reduced gonadal steroid output could readily account for the reduced LHRH action reported

in our earlier study (2). The present observations suggest that the effect of the Furth MtTW15 tumor on gonadotropin release involves a reduction of LHRH release from the hypothalamus. We believe these changes are due to the hyperprolactinemia developed in these animals.

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